

## Variation in E<sup>rns</sup> viral glycoprotein associated with failure of immunohistochemistry and commercial antigen capture ELISA to detect a field strain of bovine viral diarrhea virus

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### Abstract

Bovine viral diarrhea virus (BVDV) affects cattle populations causing clinical signs that range from subclinical immunosuppression to severe reproductive and respiratory problems. Detection and removal of persistently infected (PI) calves is the single most important factor for control and eradication of BVDV. Current testing strategies to detect PI calves rely heavily on immunohistochemistry (IHC) and a commercially available antigen capture ELISA (ACE) assay. These viral assays depend on 1 or 2 monoclonal antibodies which target the E<sup>rns</sup> glycoprotein of BVDV. The sensitivity and specificity of these two tests have been reported previously. The purpose of this research was to characterize a strain of BVDV (AU501) that was undetectable using IHC and ACE based on a single monoclonal antibody, but was consistently detected in samples from a Holstein steer using virus isolation and PCR testing. Sequencing of this AU501 viral isolate revealed a unique mutation in the portion of the genome coding for the E<sup>rns</sup> glycoprotein. This unique field strain of BVDV demonstrates the risk of relying on a single monoclonal antibody for detection of BVDV. Multiple testing strategies, including polyclonal or pooled monoclonal antibodies that detect more than one viral glycoprotein may be necessary to detect all PI calves and facilitate eradication of BVDV.

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### 1. Introduction

Bovine viral diarrhea virus (BVDV) is a respiratory and reproductive pathogen of cattle (Grooms, 2004).

This prototype of the pestivirus genus is similar to border disease virus and classical swine fever virus (Ridpath, 2005). There are two species and multiple subspecies among BVDV strains. Diversity among BVDV strains can be characterized by viral nucleotide sequence comparison or by monoclonal antibody serotyping (Ridpath et al., 1994, 2000). Type 1a, 1b and 2a are the recognized species and subspecies of BVDV circulating in North America. In addition to genotype, each strain of BVD exhibits a cytopathic or noncytopathic biotype, depending on its effect on cultured cells (Ridpath, 2005).

Bovine viral diarrhea virus is known to persist in cattle populations via animals persistently infected (PI) with BVDV. When a noncytopathic BVDV strain is transmitted through the dam to the fetus before 125–182 days of gestation, the developing bovine fetus can become immunotolerant to the infecting strain of BVDV resulting in a life long persistent infection (McClurkin et al., 1984; Ellsworth et al., 2006). Persistently infected cattle shed large quantities of BVDV throughout their life and serve as an effective source of viral transmission to other cattle as well as to other species (Ames, 2005; McClurkin et al., 1984; Fulton et al., 2006).

On the cow–calf production unit, PI calves are commonly the source of BVDV that causes acute infections in other calves and pregnant cows in the herd (Grooms and Keilen, 2002). These infected pregnant cows may give birth to PI calves during the next calving season. Persistently infected animals are also a significant concern in stocker and feedlot operations where they expose other cattle to BVDV (Larson et al., 2005). The resulting acute infections with BVDV are associated with immunosuppression that allows opportunistic pathogens to cause disease and reduce profits (Chase et al., 2004; Houe, 1999, 2003; Gunn et al., 2004).

At birth or upon entry to the stocker/feedlot operation, calves are commonly tested for persistent infection with BVDV using immunohistochemistry (IHC) or antigen capture ELISA (ACE) assays (Fulton et al., 2006). Testing of serum or whole blood from young animals may be confounded by the presence of maternal antibodies against BVDV. However, maternal antibodies do not interfere with detection of virus in skin biopsies (ear notches) and thus the ear notch has become the sample of choice for testing young

animals (Kuhne et al., 2005; Grooms and Keilen, 2002). Immunohistochemistry uses the monoclonal antibodies 15c5 (Grooms and Keilen, 2002) or 3.12F1 (Blas-Machado et al., 2004) to detect the antigen in formalin-fixed paraffin-embedded tissue. An antigen capture ELISA kit (IDEXX HerdChek BVDV Antigen ELISA—Ear Notch and Serum, IDEXX Laboratories, Inc., Westbrook, ME) that is commercially available in the United States is also based on the 15c5 monoclonal antibody to capture the BVDV antigen. Both the 15c5 and the 3.12F1 monoclonal antibodies bind the E<sup>rns</sup> viral glycoprotein, which is thought to be highly conserved among BVDV strains (Haines et al., 1992; Ridpath, 2005).

Other diagnostic tests are available and include many variations of virus isolation and polymerase chain reaction (PCR). Virus isolation and PCR are useful for detection of cattle PI with BVDV, but are slow in producing results and are technically challenging and laborious to perform (Belak and Ballagi-Pordany, 1993; Yap et al., 1994). Recent studies suggest that IHC and ACE results correlate with virus isolation (Cornish et al., 2005; Kuhne et al., 2005). The purpose of this study was to characterize a strain of BVDV (AU501) that was undetectable using IHC and ACE based on the 15c5 and 3.12F1 monoclonal antibodies, but was consistently detected in samples from a Holstein steer using virus isolation and PCR testing.

## 2. Materials and methods

### 2.1. Medical history and necropsy findings

The Holstein steer was submitted for testing based on chronic failure to thrive as indicated by height and weight compared to cohorts. Upon euthanasia of the steer at 14 months of age, necropsy findings were compiled to complete the clinical assessment.

### 2.2. Virus isolation

Serum and peripheral blood leukocyte (PBL) samples obtained over a period of 7 months (Table 1) from this calf were subjected to virus isolation by two different methods as previously described (Brock et al., 2006; Givens et al., 2003). One method utilized two monoclonal antibodies, D89 and 20.10.6 (Givens et al.,

Table 1

Results of assays to detect bovine viral diarrhea virus in samples from a Holstein steer

Age	Sample	Test	Antibody used	Epitope targeted	Result
7 months 11 days	Serum	PCR			Positive
	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
7 months 17 days	Serum	VI	D89 and 20.10.6	E2 and NS3	Positive
8 months 2 days	Serum and PBL	VI	B224 polyclonal	Multiple	Positive
	Ear notch	IHC	15c5	E <sup>tns</sup>	Negative
	Ear notch	ACE	15c5	E <sup>tns</sup>	Negative
8 months 18 days	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
8 months 19 days	Ear notch	IHC	3.12F1	E <sup>tns</sup>	Negative
9 months 1 day	Ear notch	ACE	15c5	E <sup>tns</sup>	Negative
	Serum and PBL	ACE	15c5	E <sup>tns</sup>	Negative
	Serum and PBL	VI	B224 polyclonal	Multiple	Positive
9 months 16 days	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
10 months 3 days	Ear notch	IHC	15c5	E <sup>tns</sup>	Negative
10 months 14 days	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
11 months 17 days	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
12 months 12 days	Serum and PBL	VI	D89 and 20.10.6	E2 and NS3	Positive
14 months	Serum, spleen, liver, lung, kidney and thymus	VI	D89 and 20.10.6	E2 and NS3	Positive
	Serum and PBL	PCR			Positive
	Serum and ear notch	ACE	15c5	E <sup>tns</sup>	Negative
	Serum and ear notch	ACE	Pool of monoclonals	E <sup>tns</sup>	Positive
	Ear notch	Cryo IHC	D89	E2	Positive

PBL: peripheral blood leukocytes, VI: virus isolation, ACE: antigen capture ELISA, and IHC: immunohistochemistry.

2003). The monoclonal antibody D89 is specific for E2, a major envelope glycoprotein of BVDV; 20.10.6 is specific for NS3, a conserved nonstructural protein (Vickers and Minocha, 1990; Xue et al., 1990; Corapi et al., 1990). The second method used a polyclonal antibody, B224 (Brock et al., 2006). Upon euthanasia at 14 months of age, serum, spleen, liver, lung, kidney and thymus from the calf were assayed by virus isolation using the pooled monoclonal antibody assay.

To quantitate BVDV in serum samples from the Holstein calf, serum was serially diluted (1/10) seven times and assayed in triplicate by adding 50 µL of culture medium containing Madin Darby Bovine Kidney (MDBK) cells to 90 µL of sample in a 96-well culture plate (Givens et al., 2003). The cell culture infective doses (50%, CCID<sub>50</sub>)/mL of serum were determined by the statistical method of Reed and Muench (1938).

### 2.3. Immunohistochemistry

#### 2.3.1. Formalin-fixed paraffin-embedded sections

Immunohistochemistry performed on formalin-fixed tissue from the Holstein steer utilized the

monoclonal antibodies, 15c5 or 3.12F1 as previously described (Brodersen, 2004; Blas-Machado et al., 2004). Two histopathology laboratories (University of Nebraska, Lincoln, NE and Auburn University, Auburn, AL) conducted the immunohistochemical assays on tissues. University of Nebraska Diagnostic lab performed IHC using 15c5 on ear notch samples on two different sampling dates (approximately 8 and 10 months of age; Table 1). Auburn University's histopathology lab performed IHC using 3.12F1 on ear notches obtained at 8 months 19 days of age (Table 1). Parallel sections of specimens were stained using primary antibody dilution buffer to control for nonspecific staining.

#### 2.3.2. Cryostat snap frozen sections

Ear notch sections were taken at euthanasia (Table 1) and snap frozen in blocks of Neg-50 frozen section medium (Microm International, Waldorf, Germany) using liquid nitrogen. The blocks were stored at –80 °C until being sectioned. Sections 8 µm thick were cut and placed onto slides. Slides were stored at –80 °C until staining. Tissue sections on slides were fixed for 5 min with acetone. Slides were then allowed to dry at room

temperature. Next, the primary monoclonal antibody D89 (1:500) was applied and allowed to stand on sections for 30 min. Slides were rinsed three times with Tween 20 (1:2000) and the secondary conjugated antibody (1:200; rabbit anti-mouse IgG) was applied to the sections for 30 min. After three rinses, fresh fast red chromagen (Vector Nova Red, Vector Labs, Burlingame, CA) was applied for 1.5 min and removed by rinsing the slides. Slides were counterstained with hematoxylin (Mayer's hematoxylin, DAKO North America, Carpinteria, CA), rinsed with distilled water, allowed to dry and cover slipped. Parallel sections of specimens were stained using primary antibody dilution buffer to control for nonspecific staining.

#### 2.4. Antigen capture enzyme-linked immunosorbent assay

Antigen capture ELISA (IDEXX HerdChek BVDV Antigen ELISA—Ear Notch and Serum [commercially available in the United States], IDEXX Laboratories) was performed by Auburn University and IDEXX Laboratories (Westbrook, ME) on ear notches, serum and PBL samples collected on multiple sampling dates (Table 1). This assay depends on the 15c5 monoclonal antibody to capture BVDV for detection. Ear notch and serum samples collected at Auburn University were placed into serum collection tubes at the sampling location. After transportation back to the laboratory, the ear notches were placed in phosphate buffered saline (PBS) or maintained dry at  $-20^{\circ}\text{C}$ . Serum, PBL and ear notch samples were stored according to the manufacturer's instructions. Testing was performed following the manufacturer's protocol to detect BVDV antigen using serum, PBL and ear notch samples. Serum and ear notch samples were collected upon euthanasia of the Holstein steer at 14 months of age, transported and tested by IDEXX Laboratories using this ACE.

Another antigen capture ELISA (IDEXX HerdChek BVDV Antigen ELISA—Serum [commercially available in Europe], IDEXX Laboratories) was also performed by IDEXX Laboratories (Westbrook, ME) on ear notches and serum samples collected at euthanasia (Table 1). This assay depends on a pool of monoclonal antibodies (not including 15c5) directed against the E<sup>ns</sup> glycoprotein to capture BVDV for detection. Testing was performed following

the manufacturer's protocol to detect BVDV antigen using serum and ear notch samples. Serum and ear notch samples were collected upon euthanasia of the Holstein steer at 14 months of age, transported and tested by IDEXX Laboratories using the ACE.

#### 2.5. Antigenic characterization

Antigenic characterization of the AU501 viral isolate was done at Ames, Iowa using monoclonal antibody binding assays specific for the E2 glycoprotein. Monoclonal antibody binding was assessed by indirect immunoperoxidase staining as previously described (Ridpath et al., 2000). Results of monoclonal antibody activity were compared to results with BVDV type 1 (NY-1 and TGAN) and BVDV type 2 (890 and 296nc) strains to assess the binding of diverse monoclonal antibodies to the E2 glycoprotein. The AU501 isolate was further characterized at Auburn, Alabama by indirect immunoperoxidase staining (Givens et al., 2003) with a panel of antibodies, both monoclonal and polyclonal. Antibody binding to AU501 in this assay was compared with binding to seven other type 2 strains of BVDV (296nc, 890, 1373, Parker, Arizona Spleen, CD87, and PA131) in simultaneous assays. The antibodies used in this assay were D89, 20.10.6, 15c5, 3.12F1, and a polyclonal. While D89 targets the E2 glycoprotein and 20.10.6 targets the NS3 nonstructural protein, 15c5 and 3.12F1 target epitopes in the E<sup>ns</sup> glycoprotein.

#### 2.6. Reverse transcription-nested polymerase chain reaction

A reverse transcription-nested PCR specific for the 5' nontranslated region of the BVDV genome (Givens et al., 2000) was used to detect BVDV in serum and PBL samples.

#### 2.7. Genome sequencing

To prepare RNA template, subconfluent monolayers of MDBK cells were inoculated with serum obtained from the steer at 14 months of age. Three days after inoculation, cultures, including cells and culture media, were harvested by freezing at  $-80^{\circ}\text{C}$  and thawing. An aliquot of this cell culture lysate was sent to the National Animal Disease Center (NADC) at

Ames, Iowa where PCR amplification, sequencing and analysis was performed using methods similar to a previous description (Ridpath et al., 2006). For this research, the viral genome was sequenced from nucleotide position 108–3695 (Table 2) which includes the RNA sequence that is translated into the nucleocapsid protein (C) and the three envelope glycoproteins (E<sup>ns</sup>, E1, E2) (Ridpath, 2005). Viral RNA was amplified with an RT-PCR reaction as previously described (Ridpath and Bolin, 1998). Sequences were derived by direct sequencing of RT-PCR amplification products (Ridpath et al., 2006). All sequencing reactions were done in duplicate and all sequences were confirmed by sequencing both strands. The sequence of PCR primers and the region of the genome amplified are shown in Table 2. Primers

used for sequencing were identical to those used in the primary PCR. Sequences were aligned and compared as previously described (Ridpath et al., 2006).

### 3. Results

#### 3.1. Medical history and necropsy findings

A male Holstein calf was born on 21 September 2004. The calf's dam was hospitalized between days 50 and 70 of gestation due to severe proliferative digital dermatitis. After birth, the calf was housed in a calf hutch and group housing until identified by virus isolation as PI with BVDV at 8 months of age. Testing for BVDV was warranted because of failure to thrive as indicated by height and weight. When identified as PI, the calf was moved to a pasture for BVDV PI cattle at Auburn University (Auburn IACUC #2004-0746). There he was maintained and monitored until he was euthanized at 14 months of age due to declining health. A hematology profile and blood chemistry panel during this time did not indicate any values outside of the reference interval. Samples were collected on a regular basis to monitor his BVDV status. Necropsy findings included chronic, diffuse, moderate enterocolitis and lymphadenitis. Purulent, subacute, diffuse pneumonitis and purulent, mild glomerulonephritis indicated terminal septicemia.

#### 3.2. Virus isolation

Virus isolation results from initial serum and PBL samples collected at 7 months of age were positive for BVDV. Multiple consecutive monthly samples of serum and PBL were also positive by virus isolation (Table 1). At the time of euthanasia, spleen, liver, lung, kidney and thymus were positive for BVDV using virus isolation. Titers of BVDV in serum ranged from  $3.5 \times 10^4$  to  $6.2 \times 10^4$  CCID<sub>50</sub>/mL during the sampling period.

#### 3.3. Immunohistochemistry

##### 3.3.1. Formalin-fixed paraffin-embedded sections

Immunohistochemistry using 15c5 and 3.12F1 monoclonal antibodies on ear notch samples from the Holstein steer failed to detect BVDV antigen on attempts at both laboratories (Table 1 and Fig. 1).

Table 2  
Primers used to generate sequence information

	Nucleotide position <sup>a</sup>
Forward primers	
CAT GCC CAT AGT AGG AC	108
CAC AGC CTG ATA GGG TG	320
CTA CCA CAT CAA CGA GG	524
TGC GAA TGG AGC GGT AAG	543
TGG TAT	
GCC CGT CTA TCA TAG AG	670
GAA GCA GCC TAA GCC AGA TA	922
GTT TGC ACG GAA TTT GGC	1280
CAG AGA	
GAT GGG CCC TGT AAC TT	1650
GAC TGG TGG CCA TAT GA	2255
ACT GGC TGT GCA CGA GAG AG	2653
GTA AGT GGT GTG GCT ATG AC	3076
Reverse primers	
CCA TGT GCC ATG TAC AG	386
GCA GCT GGT GAC CCA TA	889
TTC CTT CGG TGC CAT TGT CC	1241
GGT ACT CCG GTG CAA AT	1320
CCT TTG CAA TCT GCA GCA	1360
GGT GTA	
GGT CTT CTC ACT CGC AT	1387
GCC GCC GAA TTC CTA CAT CAG	1477
CCA TAT CCA CGG T	
CTA CCC TTG CTC CCT CA	1781
GCA TCA GCC ACA ATA GC	2442
CAT CTG GAA AGC TGG TC	2838
GGT ACC GGT TGG AAC TA	3215
TCC TCA CCA GTT CCT CT	3695

<sup>a</sup> Genomic location based on published sequence of the non-cytopathic, low virulence, BVDV1a strain SD-1.

### 3.3.2. Cryostat snap frozen sections

Virus was detected in ear notch sections from the steer using D89 antibody on frozen sections of ear. Staining was most intense in the apocrine glands of the skin (Fig. 1).

### 3.4. Antigen capture enzyme-linked immunosorbent assay

A positive relative optical density value was never obtained for AU501 using the ACE commercially available in the United States. Both laboratories, Auburn and IDEXX, failed to detect antigen in any of

the samples processed from the steer using this assay (Table 1). A positive relative optical density value was obtained by IDEXX laboratories using the ACE commercially available in Europe that includes a pool of monoclonal antibodies to capture BVDV (Table 1).

### 3.5. Antigenic characterization

The pattern of monoclonal antibody binding to AU501 is similar to other known type 2 BVDV isolates tested with the same panel of antibodies (Table 3). In further characterization, all antibodies bound to all viral isolates used in the immunoperoxidase mono-

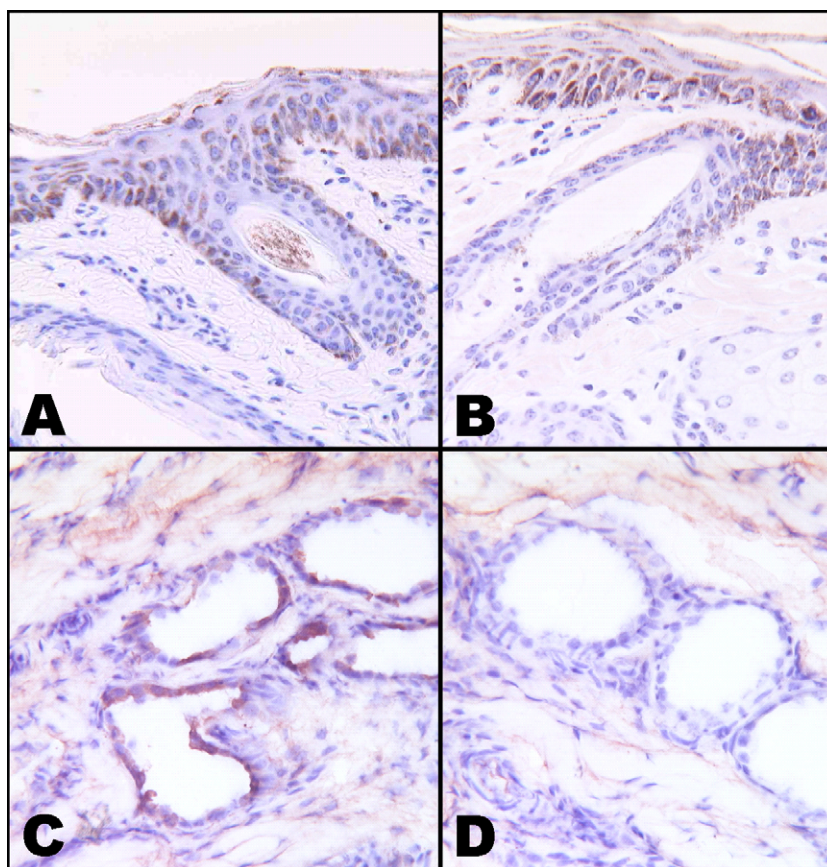


Fig. 1. Representative photomicrographs of sections of ear notch tissue obtained from the calf persistently infected with the AU501 strain of BVDV and subjected to immunohistochemical analysis for detection of BVDV (A and C) or maintained as negative controls (B and D). Sections A and B were subjected to formalin fixation, embedded in paraffin, stained with 3.12F1 primary monoclonal antibody specific for the E<sup>ms</sup> glycoprotein (A) or diluent (B), fast red chromagen substrate, and hematoxylin counterstain. Sections C and D were subjected to cryostat preservation, stained with D89 primary monoclonal antibody specific for the E2 glycoprotein (C) or diluent (D), fast red chromagen substrate, and hematoxylin counterstain. Results for section A are negative, because stain is not observed in the biopsy specimen. Results for section C are positive because there is staining of the apocrine glands of the skin.

Table 3  
Antigenic characterization using monoclonal antibodies specific for the E2 glycoprotein

Viral strain	Monoclonal antibodies																			
	CA-82	CA-80	CA-2	CA-36	CA-34	CA-3	CA-1	BZ-33	BZ-32	BZ-30	BZ-26	BZ-25	BZ-24	BZ-82	BZ-81	BZ-79	BZ-75	BZ-73	BZ-72	BZ-71
296nc	–	–	w	–	++	–	–	–	–	–	–	w	+	+	w	w	–	+	–	w
890	–	–	+	–	++	–	–	w	–	–	–	w	w	w	+	+	–	+	–	w
NY-1	w	–	+	w	++	+	+	+	–	w	–	–	++	–	–	–	–	–	–	–
TGAN	w	–	–	–	++	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–
AU501	–	–	+	–	++	–	–	–	–	–	–	–	–	+	+	+	–	+	–	w

Monoclonals CA-82–BZ-24 were previously prepared against BVDV1. Monoclonals BZ82–BZ-52 were previously prepared against BVDV2. No antibody binding (–), weak antibody binding (w), antibody binding (+), and strong antibody binding (++).

Table 4

Antigenic characterization using 1 polyclonal and multiple monoclonal antibodies for BVDV

Viral strain	Antibodies				
	D89 E2 <sup>a</sup>	20.10.6 NS3 <sup>a</sup>	15c5 E <sup>rms</sup> a	3.12F1 E <sup>rms</sup> a	B224 Poly <sup>a</sup>
296nc	+	+	+	+	+
890	+	+	+	+	+
1373	+	+	+	+	+
Parker	+	+	+	+	+
Arizona Spleen	+	+	–	–	+
CD87	+	+	+	+	+
PA131	+	+	+	+	+
AU501	+	+	–	–	+

No antibody binding (–) and antibody binding (+).

<sup>a</sup> Specific epitope.

layer assay, except antibodies targeted to the E<sup>rms</sup> glycoprotein which did not bind to AU501 or the Arizona Spleen isolate (Table 4).

### 3.6. Reverse transcription-nested polymerase chain reaction

The reverse transcription-nested PCR for BVDV yielded positive results using serum from the steer at 7 and 14 months of age (Table 1).

### 3.7. Genome sequencing

The deduced amino acid sequence for all structural proteins clearly indicates that the AU501 strain is similar to other type 2 BVDV strains (Fig. 2). However, the sequence coding for the E<sup>rms</sup> glycoprotein of AU501 indicates a mutation at nucleotide position 1330 that results in substitution of a hydrophobic, aromatic amino acid, phenylalanine, for a hydrophobic, aliphatic amino acid, leucine, at position 315 (Figs. 3 and 4). This appears to be a unique substitution not reported in any other BVDV sequences (Fig. 4). The Arizona Spleen isolate does not exhibit this unique substitution but does exhibit other unique mutations within the E<sup>rms</sup> glycoprotein (Fig. 4).

## 4. Discussion

This research involves a calf that was initially defined as PI with BVDV due to multiple positive

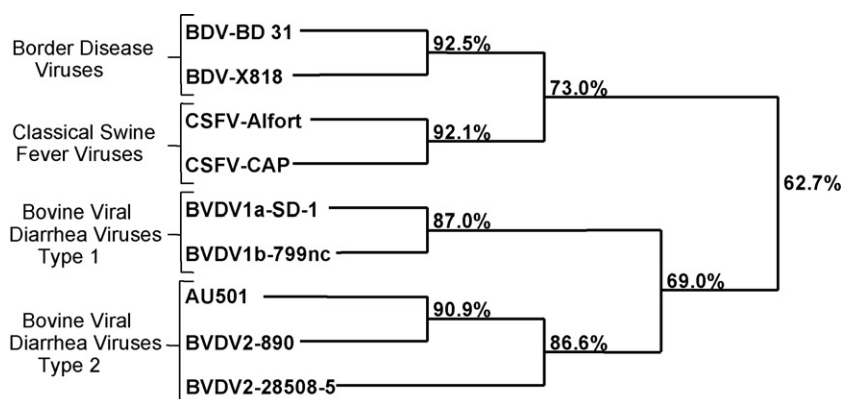


Fig. 2. A phylogenetic analysis of bovine viral diarrhea viruses and border disease viruses derived from a comparison of the deduced amino acid sequence for all structural proteins. The calculated matching percentages are indicated at each branch point of the dendrogram.

results using virus isolation and PCR. When samples from this calf failed to contain detectable virus using established methods for IHC and ACE, further investigation of this case and viral isolate was warranted due to the emphasis placed on IHC and ACE screening to identify PI calves within herds (Cornish et al., 2005).

The characteristics of the PI calf described in this study are consistent with published descriptions of clinical presentation, duration of viral detection and concentration of virus in serum (Brock et al., 1998;

Larson et al., 2004). Hospitalization of the dam because of lameness between days 50 and 70 of gestation created an opportunity for exposure to ill animals which might have transmitted BVDV to the dam and consequently to the developing fetus. Other than the inability to detect the infecting strain of BVDV using IHC or ACE, the characteristics of this PI calf are unremarkable.

This is the first report of a field strain of BVDV that cannot be detected with methods using monoclonal antibodies 15c5 and 3.12F1 that are specific for the E<sup>rms</sup> glycoprotein. This targeted glycoprotein is highly conserved and uniquely detectable after formalin fixation (Haines et al., 1992). Consequently, the E<sup>rms</sup> glycoprotein contains the principal epitopes used for detection of BVDV strains (Elahi et al., 1997). In a previous study, the 15c5 monoclonal antibody consistently detected 70 of 70 BVDV isolates and 44 of 45 cases previously diagnosed as mucosal disease (Haines et al., 1992). The single divergent result was attributed to a possible prior misdiagnosis or prolonged fixation of tissue in formalin (Haines et al., 1992). The lack of antibody binding to the E<sup>rms</sup> envelope glycoprotein of AU501 is associated with a unique mutation at amino acid position 315. This mutation is not found in any reported sequence of bovine viral diarrhea virus or border disease virus. The unique mutation within AU501 does result in an amino acid sequence reported in two isolates of classical swine fever virus (GenBank accessions AY568569.1 and AY526728.1). The binding of monoclonal antibodies to the E2 glycoprotein and the deduced

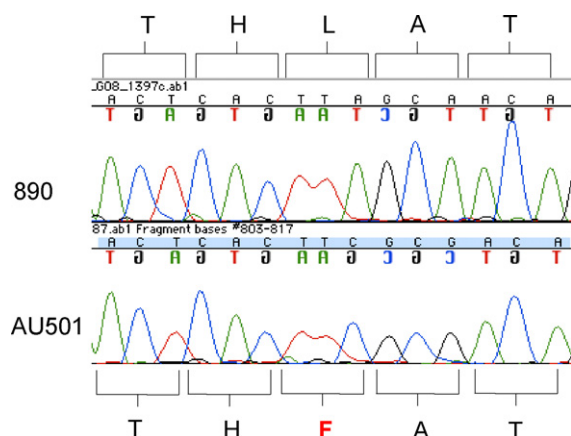
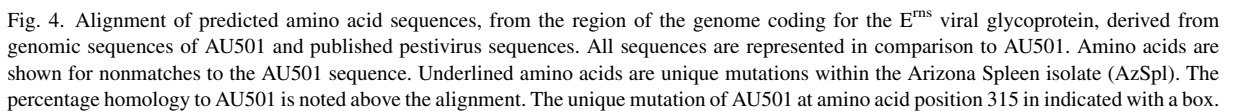


Fig. 3. An alignment of the nucleotide sequence (positions 1322–1336) and deduced amino acid sequence (positions 313–317) of the E<sup>rms</sup> viral glycoprotein of isolates AU501 and BVDV2-890 indicating a unique mutation at nucleotide position 1330 and amino acid position 315. The designation of nucleotide and amino acid positions is based on the published sequence of SD-1 (Deng and Brock, 1992).



In this study, cryostat sections from the Holstein steer were successfully stained to demonstrate BVDV antigen within ear notch biopsies of skin. This method of cryostat preservation and immunohistochemical staining for BVDV was found to maintain the histologic appearance of skin while avoiding the disadvantages of formalin fixation. This method of BVDV detection would require extensive validation before use as a routine diagnostic assay. The unique viral strain described in this research was also detected in serum and ear notch samples using the ACE commercially available in Europe which is based on a pool of monoclonal antibodies for viral capture.

As the cattle industry implements control and eradication programs for BVDV, one must realize that current IHC and ACE methods based on a single monoclonal antibody will not detect BVDV in every PI calf roaming the pastures. In contrast to previous work, this research demonstrates that the negative predictive value of IHC and ACE is less than 100% (Brodersen, 2004; Grooms and Keilen, 2002; Cornish et al., 2005; Fulton et al., 2006). Current IHC and ACE tests are useful for early detection and removal of PI calves, but should not be used as the only means to eradicate BVDV within a large population of cattle (Larson et al., 2005). Further investigation is needed to revise methods for detection of BVDV in young PI calves. Revised methods might include the use of pooled monoclonal antibodies, polyclonal antibodies, antigen retrieval systems and cryostat preservation in IHC or ACE protocols. Programs to control and eventually eradicate BVDV will be unsuccessful without the continual evaluation and development

of diagnostic assays to detect circulating field strains of BVDV.

In conclusion, this research demonstrates that a unique mutation in the E<sup>rms</sup> envelope glycoprotein is associated with failure of IHC and ACE assays based on a single monoclonal antibody to detect a type 2 field strain of BVDV that is capable of replication and persistent infection. Additional research is needed to ensure that common diagnostic methods are revised to optimize detection of all field strains of this mutable pathogen.

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